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(57) Abstract

A dicarboxylic acid hemiester or hemiamide with a pharmacologically active compound and with hyaluronic acid or a hyaluronic total or partial ester, a process for its preparation and relative controlled release medicaments containing this hemiester or hemiamide.

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A DICARBOXYLIC ACID HEMIESTER OR HEMIAMIDE WITH A PHARMACOLOGICALLY ACTIVE COMPOUND AND WITH HYALURONIC ACID OR WITH A HYALURONIC ACID ESTER, A PROCESS FOR ITS PREPARATION AND A CONTROLLED RELEASE MEDICAMENT CONTAINING THIS DERIVATIVE.

FIELD OF THE INVENTION

The present invention relates to a hemiester or a hemiamide of a dicarboxylic acid with a pharmacologically active compound and with hyaluronic acid or a hyaluronic acid total or partial ester and the relative controlled release medicament containing this derivative.

TECHNOLOGICAL BACKGROUND

Hyaluronic acid is a linear polysaccharide, whose structure is constituted by alternate units of 1,4-β-D-glucuronic acid and 1,3-β-N-acetyl-D-glucosamine.

Hyaluronic acid is a fundamental component of the connective tissue of animals as it is present, for example, in the skin and cartilage. It is also found in greater quantities in the synovial fluid, in the vitreous humor of the eye and in the umbilical cord. The main sources of commercially available hyaluronic acid at present are cockscombs and the vitreous humor. The biotechnological production of hyaluronic acid from Streptococcus cultures is becoming increasingly important. Being a fundamental component of the connective tissue, hyaluronic acid is biocompatible, bioabsorbable and not immunogenic, and it therefore plays a crucial role in many biological functions such as tissue hydration; proteoglycan organization in the cartilage, tissue repair, embryonic development, as well as the lubrication and

protection of the joint cartilage. This polysaccharide is currently used in the treatment of some joint diseases such as rheumatoid arthritis. It is also used in the practice known as microviscosurgery, in particular in surgery to the eye. When used in this field, its rheological characteristics are exploited as well as the biocompatibility of high-molecular weight hyaluronic acid. Its biological functions are closely linked with its molecular weight as they depend on the viscoelastic and rheological properties which the polymer lends to solutions (T. C. Laurent et al., The Faseb Journal, 6: 2397-2404, 1992).

Lastly, considering its excellent characteristics of biocompatibility, hyaluronic acid represents the preferred carrier in which biologically active molecules can be incorporated in controlled release systems. The properties of this material and the environmental factors determine the release rate of the active molecule.

Controlled release systems for drugs have aroused a great deal of interest in the scientific world due to their enormous applicative potential. Of the various sectors in which they can be used, the pharmaceutical field is one of the most important, and one in which such systems must needs be constantly improved to ensure optimum diffusion of the drug in the desired application site. Generally, most of these slow release systems consist of a physical mixture of active compound and suitable polymer matrix. Different kinds of controlled release formulations are, however, now coming to light, in which the drug is covalently linked to the matrix and the release rate is, in this case, modulated by the hydrolysis rate of the chemical link between the polymer and the drug.

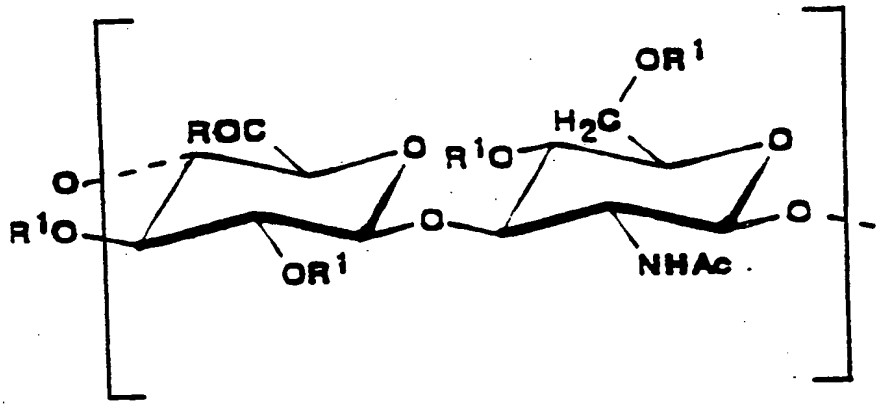
Polymers such as starch are already known to the state of the art and are used as matrices to link drugs (Ferrati et al., Macromol. Chem. 180: 375-380, 1979). For example, the treatment of succinic esters of starch with 4-aminobenzoic acid or with L-DOPA (3,4-dihydroxyphenylalanine) in N,N-dimethylformamide or dimethylsulfoxide in the presence of imidazole and N,N-dicylohexylcarbodiimide, gives a polymer derivative wherein the drug is covalently linked to the starch matrix by means of a succinate group (P. Ferrati et al., Macromol. Chem., 180 (1979) 375-380). There are, however, no known compounds of this type wherein the polysaccharide is hyaluronic acid, although there are some known systems constituted by hyaluronic acid derivatives to which the drug is chemically linked (US 4,851,521) by means of the carboxylic function of hyaluronic acid.

DESCRIPTION OF THE INVENTION

The present invention describes derivatives wherein the drug is not directly linked to the polysaccharide but is chemically reacted with a "spacer arm" before being linked to the polysaccharide. In the cases reported in the literature the position on which the substitution reaction took place was the acid group of the glucuronic residue of the repetitive unit, but in the derivatives of the present invention a reaction has been devised, whereby the positions subject to substitution are constituted by the free hydroxyl functions of the two glucuronic acid and N-acetylglucosamine residues of the repetitive unit.

A subject of the present invention therefore relates to a dicarboxylic acid hemiester or hemiamide with a pharmacologically active compound and with hyaluronic acid or a hyaluronic acid partial

or total ester having the following repeating unit (I):



(I)

wherein R = OH, an alcoholic residue or O^-Na^+, R^1 = H or $-CO(CH_2)_n-COR^2$, wherein n is an integer ranging from 1 to 10, and R^2 is an alcoholic or an aminic residue of said pharmacologically active compound.

In the hemiester, subject of the present invention hyaluronic acid can be linked to a greater quantity of drug by the introduction of a "spacer arm", constituted by the dicarboxylic acid.

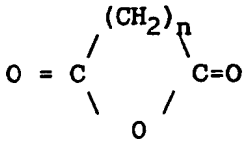
Hyaluronic acid was chosen from among the various biocompatible polysaccharides available, because of its particularly interesting properties. As we have said, its presence in various body compartments makes it far more suitable than other polysaccharides for the development of new drug matrices. Lastly, it should also be remembered that hyaluronic acid has proved therapeutically efficacious as an agent in reepithelialization, in tissue repair and in the recovery of normal conditions in joints affected by processes of an arthritic type. The use of this polymer as a matrix for drug release, apart from

guaranteeing the biocompatibility of the formulation, enables the intrinsic activity of hyaluronic acid to be associated with the therapeutic efficacy of the drug linked to it.

The hyaluronic acid esters used for preparing the dicarboxylic acid hemiester or hemiamide according to the present invention are the total or partial esters with an alcohol of the aliphatic or cycloaliphatic series, which do not themselves possess a notable pharmacological action and are disclosed in USP 4,851,521, which we incorporate herewith by reference.

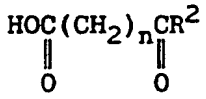
A further subject of the present invention consists in the process for preparing the dicarboxylic hemiester according to the present invention which in particular comprises the following steps:

a) reacting the dicarboxylic acid anhydride of formula (II)



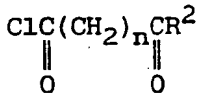
(II)

wherein n has the aforementioned meanings with stoichiometric amounts of the pharmacologically active compound of formula R^2H wherein R^2 has the above mentioned meanings in the presence of a hydrogen ion acceptor, thereby obtaining the hemiester or the hemiamide of the dicarboxylic acid with the pharmacologically active compound (III)



(III)

b) reacting the intermediate (III) coming from the preceding step with a chlorinating agent in an apolar aprotic solvent, in the presence of a catalytic amount of dimethylformamide, thereby obtaining the corresponding acyl chloride (IV):



(IV)

c) reacting the intermediate (IV) with one of the following hyaluronic acid derivatives:

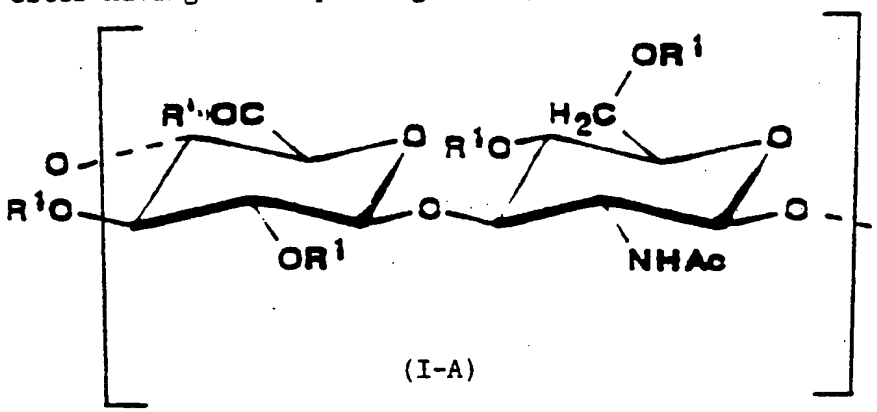
i) a salt of hyaluronic acid, whose cation is selected from: pyridinium, tetraalkylammonium, tetraarylammonium, tetraalkylphosphonium, tetraarylphosphonium,

ii) a salt of a hyaluronic acid partial ester, whose cation is selected from: pyridinium, tetraalkylammonium, tetraarylammonium, tetraalkylphosphonium, tetraarylphosphonium,

iii) a hyaluronic acid total ester,

in the presence of an aprotic solvent and an organic base as the catalyst, thereby obtaining:

the dicarboxylic acid hemiester with said pharmacologically active compound and with the salt of hyaluronic acid or a hyaluronic acid partial ester having the repeating unit (I-A)



(I-A)

wherein R'is an alcoholic residue or O^-. Y^+, wherein Y^+ is selected from the group consisting of pyridinium, tetraalkylammonium, tetraarylammonium, tetraalkylphosphonium, tetraarylphosphonium, provided that in at least one of said repeating units R' is = O^-. Y^+, or obtaining the dicarboxylic acid hemiester with said pharmacologically active substance and a hyaluronic acid total ester having the repeating unit (I) wherein R is an alcoholic residue,

d) converting by dialysis the intermediate having the repeating unit (I-A) and coming from the preceding step into the corresponding dicarboxylic acid hemiester or hemiamide having the repeating unit (I) wherein, in at least one repeating (I), R is OH or O^-Na^+.

e) recovering the desired product having the repeating unit (I) coming from the preceding step or from step (c), by freeze drying or by crystallization in an ether solvent, filtration of the desired product and successive vacuum drying. A further subject of the present invention is a controlled release medicament containing the hemiester or hemiamide according to the present invention.

DETAILED DESCRIPTION OF THE INVENTION

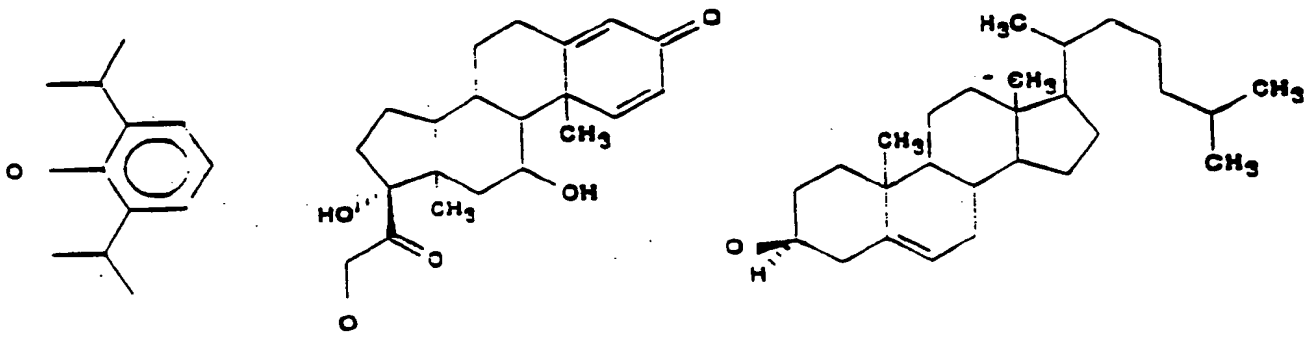
In the hemiester or hemiamide according to the present invention, when R in the repeating unit (I) is an alcoholic residue, it is preferably the residue of benzyl alcohol.

The preferred dicarboxylic acid derivatives according to the present invention are in any case the hemiesters, namely the compounds, whose repeating unit (I) has R^2 = an alcoholic residue of a pharmacologically active substance and n is preferably comprised between 2 and 4, in other words the hemiester according to the present invention comes from a dicarboxylic acid selected from the group

consisting of succinic, glutaric, and adipic acid.

Particularly preferred hemiesters according to the present invention are the succinic acid hemiesters of active substances selected from the group consisting of propofol, methyl-prednisolone (prednisone) and cholesterol.

Therefore particularly preferred hemiesters according to the present invention are those whose repeating unit (I) has R^1 = H or $CO(CH_2)_2$-COR_2 wherein R_2 is selected from



For example in the hemiester according to the present invention, when R_2 is an alcoholic residue of prednisone, hyaluronic acid is not only interesting because of its biocompatibility and bioabsorbability, but also because of its own therapeutic efficacy. In particular, the advantage of using this type of polysaccharide, substituted by a spacer arm, with compounds with antiinflammatory activity, is represented by the possibility of having slow release of the drug and of obtaining a synergic effect of the two components after hydrolysis

of the bond. Indeed, both hyaluronic acid and steroid-type antiinflammatory drugs (such as methylprednisolone) have proved to be efficacious in reducing symptoms associated with osteoarthritis of the knee (G. Leardini et al., *Clinical and Experimental Rheumatology*, 9 (1991) 375-381). Alkyl and aryl esters of hyaluronic acid have been used in preparations containing methylprednisolone, both as polymer matrices in the form of microspheres wherein the drug is physically incorporated, and as substrates on which methylprednisolone is chemically linked (K. Kyyrönen et al., *Int. J. Pharmaceutics*, 80, (1992), 161-169; L. Benedetti et al., *New Polymer Material*, 3 (1991) 41-48).

Another example of a biologically interesting molecule that has been covalently linked to hyaluronic acid by means of a spacer arm according to the present invention is represented by an antioxidant compound bearing a phenol group, i.e. the above mentioned Propofol (2,6-diisopropylphenol). In this case too, the introduction of propofol in pharmaceutical preparations containing hyaluronic acid has proved interesting, considering its activity. Indeed, the superoxide radical (O_2^-) generated by enzymatic processes can depolymerize hyaluronic acid (J. M. McCord, "Free radicals and inflammation. Protection of synovial fluid by superoxide dismutase", *Science*, 185, (1974) 529-531) thus reducing the lubricating, protective and shock absorbing properties of the synovial fluid. Phagocytosis by polymorphonucleate leukocytes produces superoxide radicals which can then constitute one of the mechanisms of degradation of the synovial fluid *in vivo* in inflammatory situations of the joints. It has been demonstrated that many antiinflammatory drugs and free radical

scavengers are efficacious in protecting hyaluronic acid from depolymerization by radicals (P. Puig-Parellada et al., *Biochem. Pharmacology*, 27, (1978) 535-537; C. Parenti et al., *Pharmazie*, 45 (1990) 680-681).

Propofol is an anaesthetic for intravenous administration whose efficacy as a free radical scavenger is due to its ability to form stable radicals. Indeed, its efficacy as a scavenger for hydroxy radicals generated by xanthine oxidase/hypoxanthine has been demonstrated by assessing *in vitro* the depolymerization of hyaluronic acid in artificial synovial fluid (C. Kvam et al., *Biochem. Biophys Res. Commun.*, 193 (1993) 927-933). For this reason, Propofol has been proposed as a possible shield for hyaluronic acid. The use of the succinic group as a spacer arm proves to be particularly important in this case because the formation of a direct ester bond between the phenol group and the carboxyl group of the polymer is particularly difficult due to steric hindrance.

In the process according to the present invention for preparing the above mentioned preferred succinic acid hemiesters having the repeating unit (I) wherein R_2 is an alcoholic residue of prednisone, propofol, cholesterol or any other molecule with an alcoholic residue step (a) is carried out by reacting succinic anhydride in the presence of an organic base such as pyridine as the hydrogen ion acceptor, to obtain the corresponding hemiester (II). In step (b) the chlorinating agent used to transform the hemiester into the corresponding chloride acyl can be any chlorinating reagent such as thionyl chloride or sulfuryl chloride. In this case we used oxalyl chloride in methylene chloride in the presence of catalytic quantities

of N,N-dimethylformamide.

Step (c) is preferably carried out by using dimethylformamide as the solvent and by using as the catalyst pyridine, 4 dimethyl amino pyridine or a mixture thereof .

To prepare the hemiester according to the present invention with antiinflammatory drugs, hyaluronic acid and the alkyl and the partial or total esters thereof, of any molecular weight, can be used. The following examples were prepared using samples of hyaluronic acid with molecular weights ranging from 30,000 to 760,000.

The succinyl derivatives of hyaluronic acid or the esters thereof conjugated with drugs such as prednisone, propofol and cholesterol etc. described in the present invention can, therefore, be used to advantage directly or in pharmaceutical formulations for the treatment of various forms of arthritis and joint inflammations.

The above-mentioned compounds constitute a probable substrate for the action of the lipases in the human body and in one case supply hyaluronic acid, succinic acid and the drug, and in the case of benzyl derivatives of hyaluronic acid they supply hyaluronic acid, benzyl alcohol, succinic acid and the drug.

For purely illustrative purposes we report hereafter some examples of the preparation of succinyl derivatives of hyaluronic acid or of its esters, covalently linked to biologically active molecules:

Example 1

Preparation of hemisuccinate propofol

A solution of Propofol (10 mL, 54 mmol) in pyridine (25 mL) was supplemented with succinic anhydride (4.86 g, 48.6 mmol) while being stirred at 70°C for 96 hours. The solution was then concentrated in

conditions of reduced pressure until a syrup was obtained. The residue was chromatographed on a silica gel column using diethyl ether and petroleum ether (low-boiling fraction) (2:3), after crystallization from diethyl ether to petroleum ether had given the hemisuccinate propofol compound (7.0 g), melting point 101-102°C.

The ^{13}C n.m.r. spectrum of the product in DMSO-d_6 (50.3 MHz) presents the following signals: d 179.8 (COOH), 171 (C=O), 145.63, 140.54, 126.79, 124.11 (aromatic carbons), 28.04, 28.83 (CH_2), 27.64 (CH), 23.38 (CH_3).

The spectrum of the proton in DMSO-d_6 presents the following signals: d 1.21, 1.23, (CH_3), 2.71-3.0 (CH_2, CH), 7.12-7.27 (aromatic protons).

Preparation of propofol-succinyl-hyaluronate

Hyaluronic acid (MW 30,000, 100 mg) was dissolved in distilled water (25 mL) and the pH was brought to approximately 2.5 using an ion exchange resin (IRA 120 H^+). The resin was then removed by filtration and the solution concentrated to about 10 mL. 50 mL of N,N-dimethylformamide (DMF) were then added and the solution was concentrated to about 20 mL. This procedure was repeated three times to substitute most of the water with DMF. The solution was then neutralized with an excess of pyridine (5 mL) to give a solution with a gel-like consistency that was then treated with a solution of propofol succinyl chloride [prepared from hemisuccinate propofol (36 mg, 0.14 mmol), DMF (1 drop), oxalyl chloride (120 µL, 0.14 mmol) in anhydrous methylene chloride (3 mL) for 1 hour] and shaken at room temperature for 24 hours. The reaction mixture was then dialyzed against distilled water (6 times 1 L). The resulting solution (opalescent) was freeze-dried to give propofol-succinyl-hyaluronate in

the form of white flakes (72 mg).

The product has limited solubility in water. The n.m.r. spectra of ^{1}H and ^{13}C show the presence of signals due to the protons and carbons of the succinic group. The spectrum confirms the presence of protons of an aromatic type. The degree of substitution based on qualitative n.m.r. results is 11-15%.

Hyaluronic acid (MW 30,000, 250 mg) was dissolved in distilled water (50 mL) and the pH was brought to approximately 2.5, using an ion exchange resin (IRA 120 H^{+}). The resin was then removed by filtration and the solution was concentrated to about 10 mL. 50 mL of N,N-dimethylformamide (DMF) were then added and the solution was concentrated to about 20 mL. This procedure was repeated three times to substitute most of the water with DMF. The solution was then neutralized with an excess of pyridine (5 mL) to give a solution with a gel-like consistency which was then treated with a solution of propofol succinyl chloride [prepared from hemisuccinate propofol (90 mg), DMF (1 drop), oxalyl chloride (300 µL) in anhydrous methylene chloride (7.5 mL)] and shaken at room temperature for 24 hours. The product was then precipitated with ether, washed with methylene chloride to remove all the reagents and vacuum-dried for 24 hours to give propofol-succinyl-hyaluronate (250 mg).

Table 1 reports the sample's chemical shifts in DMSO. In all the tables reporting n.m.r. data, G indicates the glucuronic acid residue of hyaluronic acid (eg, G-1 indicates the anomeric carbon of glucuronic acid), N represents the glucosamine residue (eg, N-6 indicates carbon 6 of the glucosamine residue).

- TABLE 1 -

Chemical shift δ ppm	non-modified HA	modified HA	other groups
101.66	N-1		
55.45	N-2		
84.10	N-3		
69.99	N-4		
76.30	N-5		
62.02	N-6		
23.88	N-Ac		
104.20	G-1		
73.67	G-2		
75.04	G-3		
81.99	G-4		
76.93	G-5		
172.90	G-6		
175.07	N=C=O		
99.10		N-1	
54.05		N-2	
82.28		N-3	
63.30		N-6	
22.92		N-Ac	
71.74		G-2	
28.52			succinic CH_2
172.03			succinic C=O
22.94, 26.71			CH_3, CH propofol
123.66, 126.23			Aromatic carbons

The degree of substitution calculated according to the esterification in N-6 was estimated at about 100%.

Example 2

Preparation of the propofol-succinyl derivative of the hyaluronic acid benzyl ester (HABE)

A suspension of HABE (MW 140,000, 250 mg) in N,N-dimethylformamide (DMF, 16 mL) was stirred at room temperature for 0.5 hours, after which were added: pyridine (5 mL), 4-dimethylaminopyridine (20 mg) and a solution of propofol succinyl chloride [prepared from hemisuccinate propofol (410 mg), DMF (2 drops), oxalyl chloride (130 µL) in ether (3 mL)]. The mixture was stirred at room temperature for 24 hours. The product was then precipitated from ether, repeatedly washed with methylene chloride to remove all the reagents used and then vacuum-dried to give the propofol succinyl derivative of the hyaluronic acid benzyl ester (250 mg).

Table 2 reports the chemical shift values for the sample in DMSO.

- TABLE 2-

Chemical shift δ ppm	non-modified HA	modified HA	other groups
101.45	N-1		
59.90	N-2		
83.51	N-3		
68.86	N-4		
76.31	N-5		
60.93	N-6		
22.94	N-Ac		
102.68	G-1		
72.60	G-2		
73.52	G-3		
80.10	G-4		
79.113	G-5		
167.29	G-6		
169.58	N=C=0		
100.16		N-1	
59.40		N-2	
81.99		N-3	
68.86		N-4	
73.18		N-5	
63.51		N-6	
79.66		G-4	
28.96			succinic CH_2
171.52, 170.78			succinic C=0
22.99, 26.72			CH_3, CH
			Propofol
123.66, 126.23			Aromatic
			carbons

It was possible to estimate the degree of substitution from the n.m.r. spectrum, according to the integration relative to carbon N-6, which was 45%.

Example 3

Synthesis of hemisuccinate cholesterol

A solution of cholesterol (7.5 g, 19.4 mmol) in pyridine (20 ml) was supplemented with succinic anhydride (2.04 g, 20.4 mmol) and the mixture was stirred at 60°C for 48 hours. The solution was then concentrated at reduced pressure to give, after crystallization from butane-2-one, hemisuccinate cholesterol (6.62 g).

Preparation of cholesterol-succinyl-hyaluronate

Hyaluronic acid (MW 30.000, 500 mg) was dissolved in distilled water (125 mL) and the pH was brought to approximately 2.5 using an ion exchange resin (IRA 120 H^+). The resin was then removed by filtration and the solution was concentrated to about 45 mL. 100 mL of N,N-dimethylformamide (DMF) were then added and the solution was concentrated to about 50 mL. This procedure was repeated three times to substitute most of the water with DMF. The solution was then neutralized with an excess of pyridine (17 mL) to give a solution with a gel-like consistency which was then treated with a solution of cholesterol succinyl chloride [prepared from hemisuccinate cholesterol (2.68 g, 5.5 mmol), DMF (1 drop), oxalyl chloride (0.51 mL, 5.8 mmol) in anhydrous methylene chloride (8 mL)] and stirred at room temperature for 24 hours. The reaction mixture was then dialyzed against distilled water to give a water-soluble fraction which was then freeze-dried (230 mg) and a water-insoluble fraction which was vacuum-dried in the presence of phosphoric anhydride to give

cholesterolsuccinyl-hyaluronate (1.5 g).

The water-soluble derivative is characterized by a lower degree of modification with cholesterol-succinate than that of the water-insoluble fraction.

Example 4

Preparation of the cholesterol-succinyl derivative of hyaluronic acid benzyl ester (HABE)

A suspension of HABE (MW 140,000, 500 mg) in N,N-dimethylformamide (DMF, 16 mL) was stirred at room temperature for 0.5 hours and then supplemented with: pyridine (4 mL), 4-dimethylaminopyridine (5 mg) and a solution of cholesterol succinyl chloride [prepared from hemisuccinate cholesterol (1.04 g, 4.29 mmol), DMF (2 drops), oxalyl chloride (0.392 mL, 45 mmol) in ether (9 mL)]. The mixture was stirred at room temperature for 24 hours. The reaction mixture was concentrated to about half the original volume and the product was precipitated from ether, gathered with ethanol to give cholesterol-succinyl-HABE in the form of a solid product (550 mg).

Table 3 reports the assignment of 13C-n.m.r. signals of cholesterol-succinylHABE.

- TABLE 3 -

Chemical shift δ ppm	non-modified HA	modified HA	other groups
100.45	N-1		
59.90	N-2		
83.51	N-3		
68.86	N-4		
76.31	N-5		
60.93	N-6		
22.94	N-Ac		
102.68	G-1		
72.60	G-2		
73.52	G-3		
80.10	G-4		
79.11	G-5		
167.29	G-6		
169.58	N=C=O		
100.00		N-1	
82.00		N-3	
63.40		N-6	
79.55		G-4	
28.70			succinic CH_2
171.55, 170.02			succinic C=O
11.57 -49.5			CH_3, CH_2, CH cholesterol
121.87, 139.47			Aromatic carbons of benzyl group

Example 5

Preparation of hemisuccinate prednisone

A solution of prednisone (750 mg, 2.1 mmol) in anhydrous pyridine (10 ml) is supplemented with succinic anhydride (314 mg, 3.14 mmol) while stirring the mixture at 60°C for 96 hours. The solution is then concentrated at reduced pressure, the residue is dissolved in methylene chloride and then placed in a silica gel column and vacuum-dried. Silica gel chromatography using methylene chloride - petroleum ether (2:1) gives hemisuccinate prednisone (650 mg).

Preparation of prednisone succinyl hyaluronate

Hyaluronic acid (MW 30,000, 500 mg) was dissolved in distilled water (125 mL) and the pH was brought to approximately 2.5 using an ion exchange resin (IRA 120 H^+). The resin was then removed by filtration and the solution concentrated to about 45 mL. 100 mL of N,N-dimethylformamide (DMF) were added and the solution was concentrated to about 50 mL. This procedure was repeated three times to substitute most of the water with DMF. The solution was then neutralized with an excess of pyridine (17 mL) to give a solution with a gel-like consistency which was then treated with a solution of prednisone succinyl chloride [prepared from hemisuccinate prednisone (280 mg, 0.58 mmol), by partially dissolving it in chloroform (3 mL) and dioxane (8 mL) and DMF (1 drop), and then adding oxalyl chloride (0.51 mL, 5.8 mmol) and stirring for 1 hour] and stirred at room temperature for 18 hours. The solution was concentrated to 20 mL, gathered with warm water (50°C) and then dialyzed against distilled water (6 times, 1 L). The prednisone-succinyl-hyaluronate was then recovered by freeze-drying (350 mg).

Table 4 shows the assignment of the ^{13}C-n.m.r. spectrum in DMSO:D_2O (1:15) of the prednisone succinyl hyaluronate.

- TABLE 4 -

Chemical shift δ ppm	non-modified HA	modified HA	other groups
101.66	N-1		
55.45	N-2		
84.10	N-3		
69.99	N-4		
76.30	N-5		
62.02	N-6		
23.88	N-Ac		
104.20	G-1		
73.67	G-2		
75.04	G-3		
81.99	G-4		
76.93	G-5		
172.90	G-6		
175.07	N=C=O		
63.50		N-6	
29.77			succinic CH_2
159.43			prednisone CO
11.58 -68.40			CH_3, CH_2, CH of prednisone

N.m.r. data show the degree of substitution of the succinyl prednisone on the polymer to be about 5%.

Example 6

Preparation of the succinyl prednisone derivative of the hyaluronic acid benzyl ester (HABE)

A suspension of HABE (MW 140,000, 100 mg) in N,N-dimethylformamide (DMF, 16 mL) was stirred at room temperature for 0.5 hours and then supplemented with: pyridine (4 mL), 4-dimethylaminopyridine (5 mg) and a solution of prednisone succinyl chloride [prepared from hemisuccinate prednisone (170 mg), DMF (2 drops), oxalyl chloride (34µL) in ether (5 mL)]. The mixture was stirred at room temperature for 24 hours. The reaction mixture was concentrated to about half its original volume and the product was then precipitated from ether, gathered with ethanol and stirred for 0.5 hours to give prednisone-succinyl-HABE (120 mg).

Table 5 shows the assignment of the ^{13}C-n.m.r. spectrum of the prednisone succinyl-HABE.

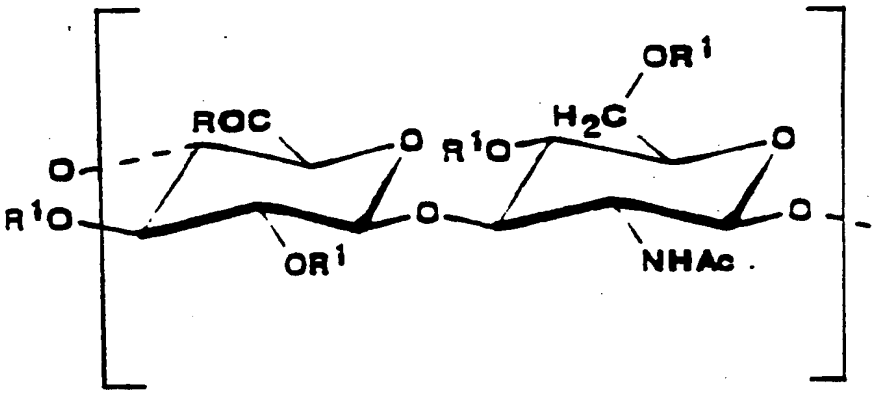
- TABLE 5 -

Chemical shift δ ppm	non-modified HA	modified HA	other groups
100.45	N-1		
59.90	N-2		
83.51	N-3		
68.86	N-4		
76.31	N-5		
60.93	N-6		
22.99	N-Ac		
102.68	G-1		
72.60	G-2		
73.52	G-3		
80.10	G-4		
79.11	G-5		
167.29	G-6		
169.58	N=C=O		
22.89		N-Ac	
82.00		N-3	
63.50		N-6	
79.70		G-4	
171.35, 166.63			succinic CH_2
154.80			CO prednisone
14.93, 49.38			CH_3, CH_2, CH
59.18, 67.71			prednisone
123.69, 135.33			Aromatic carbons of benzyl group

^{13}C-n.m.r. data show the degree of substitution of the polymer with succinyl prednisone to be about 10%.

CLAIMS

1. A dicarboxylic acid hemiester or hemiamide with a pharmacologically active compound and with hyaluronic acid or a hyaluronic acid partial or total ester having the following repeating unit (I):



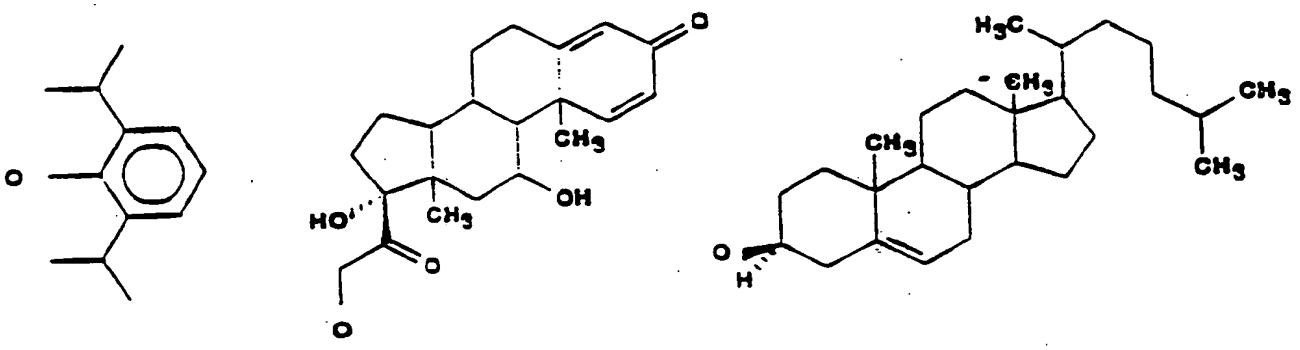
(I)

wherein R = OH, an alcoholic residue or O^-Na^+, R^1 = H or $-CO(CH_2)_n-COR^2$, wherein n is an integer ranging from 1 to 10, and R^2 is an alcoholic or an aminic residue of said pharmacologically active compound.

2. The dicarboxylic acid hemiester or hemiamide according to claim 1 wherein, when R in the repeating unit (I) is an alcoholic residue, it is the residue of benzyl alcohol.

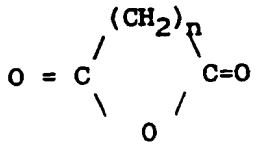
3. The dicarboxylic acid hemiester according to one of the claims 1 or 2, whose repeating unit (I) has R^2 = an alcoholic residue of a pharmacologically active substance and n is comprised between 2 and 4.

4. The dicarboxylic acid hemiester according to one of the claims 1, 2, 3 whose repeating unit (I) has R^1 = H or $CO(CH_2)_2-COR_2$ wherein R_2 is selected from the group consisting of:



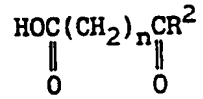
5. A process for preparing the dicarboxylic hemiester or hemiamide according to one of the claims 1-3, comprising the following steps:

a) reacting the dicarboxylic acid anhydride of formula (II):



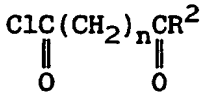
(II)

wherein n has the aforementioned meanings with stoichiometric amounts of the pharmacologically active compound of formula R^2H wherein R^2 has the above mentioned meanings in the presence of a hydrogen ion acceptor, thereby obtaining the hemiester or the hemiamide of the dicarboxylic acid with the pharmacologically active compound (III)



(III)

b) reacting the intermediate (III) coming from the preceding step with a chlorinating agent in an apolar aprotic solvent, in the presence of a catalytic amount of dimethylformamide, thereby obtaining the corresponding acyl chloride (IV):



(IV)

c) reacting the intermediate (IV) with one of the following hyaluronic acid derivatives:

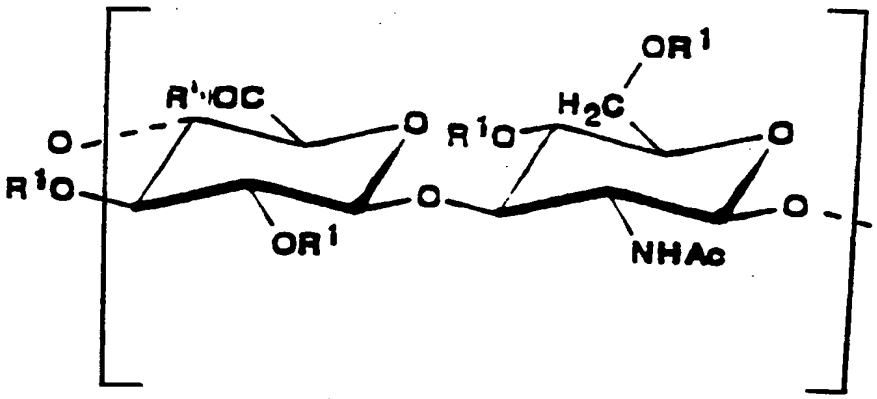
i) a salt of hyaluronic acid, whose cation is selected from the group consisting of: pyridinium, tetraalkylammonium, tetraarylammonium, tetraalkylphosphonium, tetraarylphosphonium

ii) a salt of a hyaluronic acid partial ester, whose cation is selected from the group consisting of: pyridinium, tetraalkylammonium, tetraarylammonium, tetraalkylphosphonium, tetraarylphosphonium

iii) a hyaluronic acid total ester,

in the presence of an aprotic solvent and an organic base as the catalyst, thereby obtaining:

the dicarboxylic acid hemiester with said pharmacologically active compound and with the salt of hyaluronic acid or a hyaluronic acid partial ester having the repeating unit (I-A)



(I-A)

wherein R'is an alcoholic residue or O^-. Y^+ wherein Y^+ is selected from the group consisting of pyridinium, tetraalkylammonium, tetraarylammonium, tetraalkylphosphonium, tetraarylphosphonium provided that in at least one of said repeating units R' is = O^-. Y^+, or obtaining

the dicarboxylic acid hemiester with said pharmacologically active substance and a hyaluronic acid total ester having the repeating unit (I) wherein R is an alcoholic residue,

d) converting by dialysis the intermediate having the repeating unit (I-A) and coming from the preceding step into the corresponding dicarboxylic acid hemiester or hemiamide having the repeating unit (I), wherein, in at least one repeating unit (I), R is OH or O^-Na^+,

e) recovering the desired product coming from the preceding step or from step (c), by freeze drying, or by crystallization carried out in the presence of an ether solvent, filtration of the desired product and successive vacuum drying.

6. The process according to claim 5 for preparing the succinic acid hemiesters according to claim 4, wherein step (a) is carried out by

reacting succinic anhydride in the presence of pyridine as the hydrogen ion acceptor, to obtain the corresponding hemiester (II) and by using as the chlorinating agent oxalyl chloride.

7. A process according to one of claims 5 and 6 wherein step (c) is carried out by using an organic base, as the catalyst, selected from the group consisting of pyridine, 4-dimethyl amino pyridine and a mixture thereof.

8. A controlled release medicament comprising the dicarboxylic acid hemiester or hemiamide according to one of the claims 1-4.

9. A controlled release medicament according to claim 8 containing the succinic acid hemiester according to claim 4 for the treatment of osteoarticular joint diseases.

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/EP 96/01980

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C08B37/08 A61K47/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C08B A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PATENT ABSTRACTS OF JAPAN vol. 17, no. 339 (C-1075), 28 June 1993 & JP,A,05 039306 (D D S KENKYUSHUKO), 19 February 1993, see abstract ---	1-9
Y	EP,A,0 540 751 (EISAI CO.) 12 May 1993 see examples 1-5 ---	1-9
Y	PATENT ABSTRACTS OF JAPAN vol. 18, no. 222 (C-1193), 21 April 1994 & JP,A,06 016702 (SHISEIDO CO LTD), 25 January 1994, see abstract ---	1-9

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
18 September 1996	02. 10. 96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Lensen, H

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 96/01980

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	INTERNATIONAL JOURNAL OF PHARMACEUTICS , vol. 80, 1992, pages 161-169, XP000600705 L. KYYRÖNEN ET AL.: "Methylprednisolone esters of hyaluronic acid in ophthalmic drug delivery : in vitro and in vivo release studies." cited in the application see page 162, left-hand column, paragraph 2 -----	

1

INTERNATIONAL SEARCH REPORT

...ıformation on patent family members

Interr ·onal Application No
PC1/EP 96/01980

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-540751	12-05-93	WO-A- 9220699	26-11-92

Form PCT/ISA/210 (patent family annex) (July 1992)